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THE BANGKOK SCHOOL OF TROPICAL MEDICINE
UNIVERSITY OF MEDICAL SCIENCES

FINAL PROGRESS REPORT NO. I
(THE FIRST YEAR)

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STUDIES ON LEPTOSPIROSIS IN THAILAND,
WITH SPECIAL REFERENCE TO THE EPIDEMIOLOGY,
PATHOLOGY AND CLINICAL ASPECTS, AND ITS RELATION
TO THE ANIMAL RESERVOIR HOSTS

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FINAL PROGRESS REPORT NO. I

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Title of the Project: Studies on leptospirosis in Thailand, with special reference to the epidemiology, pathology and clinical aspects, and its relation to the animal reservoir hosts.

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Period covered: 1 September 1964-31 August 1965.

Title of Projects

STUDIES ON LEPTOSPIROSIS IN THAILAND

A. Human leptospirosis

1. Isolation of leptospire from the patients
2. Serological studies of suspected cases of leptospirosis.
3. Clinical studies.
4. Surveys of leptospiral antibodies in the general population of Thailand.
5. Detection of leptospirosis in cases of pyrexia of unknown origin.
6. Macroscopic screening test of leptospirosis.

B. Leptospirosis in animals

1. The incidence of leptospirosis in rats in Bangkok.
2. Seasonal incidence of canine leptospirosis in Bangkok.
3. Studies on renal pathology in canine leptospirosis.

ABSTRACT

Studies on leptospirosis, with special reference to the epidemiology, pathology and clinical aspects, and its relation to the animal reservoir hosts

Epidemiological study on leptospirosis in Thailand by surveying leptospiral antibodies among the general population in 71 provinces revealed positive agglutination reactions of 22-75%. The high incidences were mostly found in South Thailand. The common serotypes were L.grippotyphosa, L.icterohemorrhagiae and L.bataviae. Most cases were farmers; the ratio of males to females was 3:2.

Examinations for laboratory diagnosis of leptospirosis were made in the past twelve months in about 1400 patients of various hospitals throughout Thailand. The results showed that L.bataviae was the most prevalent serotype in Bangkok, while L.grippotyphosa was the common one in the provincial areas.

The clinical and laboratory features of 34 cases of leptospirosis, most of which caused by L.bataviae, were also analysed.

About 15% of cases of pyrexia of unknown origin in a municipality hospital of Bangkok were found to suffer from leptospirosis, and L.bataviae and L.canicola were the common serotypes involved.

The results of macroscopic screening tests in human leptospirosis were satisfactory, while in animal leptospirosis only the specimens with high titres gave some positive reactions. L.biflexa No. 138 had a promising antigenic property reacted to L.bataviae antisera.

Studies on leptospirosis in rats caught from Pathumwan District of Bangkok revealed positive kidney-culture cases of 67% in adult rats and 12% in immature ones. The incidence among the adult rats in the wet season was 65% and in the dry season 60%.

The incidence of canine leptospirosis in Bangkok diagnosed by serology was found to be 64%. The results also indicated that recent infections in dogs were present mostly in the wet season.

The preliminary results of the studies of renal pathology in canine leptospirosis revealed definite pathological changes in cases of positive kidney cultures, while in cases of positive serology, only mild grades of inflammatory foci were observed. In those negative cases, however, slight histological changes were found in some animals.

A. Human leptospirosis

1. Isolation of leptospires from the patients

During the past 12 months, hemocultures and animal inoculations were tried in suspected cases of leptospirosis in three major hospitals in Bangkok i.e. Siriraj Hospital, Chulalongkorn Hospital and the Hospital for Tropical Diseases.

Results

A total of 122 cases clinically diagnosed as leptospirosis were included in this investigation. Hemoculture was performed in 98 cases, 30 of which (30.6%) gave positive results, while hamster inoculation in 29 cases, 9 of which (31.0%) were positive. The details of the results are shown in Table I.

Table I The results of hemoculture and hamster inoculation in suspected cases of leptospirosis admitted into three hospitals.

	Hemoculture			Animal inoculation		
	No. exam.	No. pos.	Serogroups of pos. <u>Leptospira</u>	No. exam.	No. pos.	Serogroups of pos. <u>Leptospira</u>
Siriraj	31	3	1 L.bataviae 1 L.canicola 1 L.ictero.	-	-	-
Chulalongkorn	33	16	15 bataviae 1 canicola	-	-	-
Tropical Diseases	34	11	8 bataviae 1 javanica 2 canicola	29	9	8 bataviae 1 javanica
Total	98	30	24 bataviae 4 canicola 1 javanica 1 ictero.	29	9	8 bataviae 1 javanica

Summary and Conclusion

Analysis of cases in regard to the epidemiology and clinical aspects is being performed.

2. Serological studies of suspected cases of leptospirosis.

Specimens of sera of suspected cases of leptospirosis from various hospitals throughout Thailand (11 hospitals in Bangkok and 80 provincial hospitals) were sent to our laboratory for conclusive diagnosis. Those include fresh sera from nearby hospitals in Bangkok and filter paper dried blood samples from provincial hospitals. Agglutination test was made by using 11 Leptospira serotypes as antigens.

Results

(i) Hospitals in Bangkok area

On examination of 513 specimens of sera, 102 (19.9%) gave positive results. However, the total positive cases were 60, as paired sera of specimens from many cases were sent for confirmation. The serotypes involved in this study were 48 L.bataviae, 6 L.canicola, 2 L.icterohemorrhagiae, 2 L.javanica, 1 L.hebdomadis and 1 L.australis A.

(ii) Provincial hospitals

786 specimens of dried blood on filter papers from 30 provincial hospitals were tested for leptospirosis; 97 (12.3%) showed positive results. The serotypes involved were identified as 34 L.grippotyphosa, 15 L.icterohemorrhagiae, 15 L.bataviae, 12 L.hebdomadis, 7 L.akiyami A, 6 L.pomona, 4 L.canicola, 2 L.australis A, 1 L.javanica and 1 L.pyrogenes.

Summary and Conclusion

Diagnosis of leptospirosis in patients of various hospitals, especially those in the provincial areas, is now facilitated by sending dried blood on filter paper to our laboratory in Bangkok, where agglutination test is made.

In Bangkok area, L.bataviae was the most prevalent serotype responsible for leptospirosis in man, while in the provincial areas L.grippityphosa was the common one.

3. Clinical studies

Another series of 34 cases of leptospirosis admitted to Siriraj, Chulalongkorn and Tropical Disease Hospitals during the last year was studied. The results are shown in Table II-VII

Table II Age and sex

Age in years	Male	Female	Total	
			No.	Per/cent
9 and lower	0	0	0	0
10 - 19	1	4	5	14.70
20 - 29	9	1	10	30.0
30 - 39	5	2	7	20.0
40 - 49	4	2	6	17.65
50 and over	5	1	6	17.65
Total	24	10	34	100

Table III The monthly incidence

Winter		Summer			Rainy season					Winter	
Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	1	-	-	4	2	-	1	2	13	7	3

Table IV The occupation

Occupation	No. patients
Labourer	18
Merchant	3
House wife	6
Farmer	3
Student	2
Government Officer	1
Poultry feeder	1

Table V The symptoms and signs

Symptoms	No.of cases	Signs	No.of cases
Injected eyes	32	Jaundice	18
Myalgia	31	Abdominal tenderness	15
Sudden onset of fever	31	Enlargement of liver	14
Anorexia	29	Photophobia	9
Headache	27	High continuous fever	9
Nausea	26	Low grade of fever	6
Vomiting	25	Constipation	6
Cough	14	Dull consciousness	6
Diarrhea	12	Petechial hemorrhage	4
Chest pain	10	Rale and rhanchi	4
Retrobulbar pain	8		

Table VI The important laboratory findings

Blood and Blood Chemistry	No. of cases	Urine	No. of cases
W.B.C., normal (6,000-10,000)	4	W.B.C.	26
		Granular casts	24
over 10,000	30	R.B.C.	20
N.P.N., normal	11	Albuminuria	19
40-100 mg%	14	Bile	9
over 100 mg%	7		

Table VII The Results of agglutination test, hemoculture and hamster inoculation among 34 cases of leptospirosis

Case No.	Titres at weekly interval				Hemoculture	Hamster inoculation
	1st	2 nd	3 rd	4 th		
1	-ve	3,000C	3,000C	10,000C	+ve C	-ve
2	-ve	10,000J	10,000J	3,000J	+ve J	+ve J
3	-ve	-	-	-	+ve B	-
4	-ve	300B	3,000B	10,000B	+ve B	+ve B
5	300B	1,000B	1,000B	10,000B	-ve	-ve
6	-ve	1,000B	10,000B	-	-	-
7	-ve	1,000B	3,000B	-	+ve B	-
8	-ve	-	-	-	+ve B	-
9	-ve	30,000B	-	-	+ve B	-
10	-ve	1,000B	30,000B	30,000B	+ve B	-
11	-ve	300B	10,000B	30,000B	+ve B	+ve B
12	-ve	300B	1,000B	-	+ve B	-
13	-ve	300B	1,000B	-	-ve	-ve
14	-ve	1,000J	10,000J	10,000J	+ve J	+ J
15	+ve	-	-	-	+ve C	-
16	-ve	-	-	-	+ve B	+ve B
17	-ve	100B	-	-	+ve B	-
18	-ve	-	-	-	+ve B	-
19	-ve	10,000B	1,000B	-	-	-
20	-ve	1,000B	30,000B	-	-ve	-

Case No.	Titres at weekly interval				Hemoculture	Hamster inoculation
	1 st	2 nd	3 rd	4th		
21	100B	10,000B	-	-	-	-
22	-ve	30,000B	-	-	+ve B	-
23	-ve	3,000B	3,000B	30,000B	-ve	-ve
24	-ve	10,000B	-	-	+ve B	-
25	-ve	3,000B	-	-	-	-
26	-ve	1,000I	-	-	-	-
27	-ve	1,000B	10,000B	-	-	-
28	100B	3,000B	-	-	-	-
29	-ve	-	-	-	+ve B	-
30	-ve	3,000B	-	-	-ve	-
31	-ve	-	-	-	+ve B	-
32	-ve	300B	1,000B	10,000B	+ve B	-ve
33	-ve	1,000B	30,000B	-	+ve B	+ve B
34	-ve	10,000B	3,000B	1,000B	-ve	-ve

B = *L.bataviae*

C = *L.canicola*

J = *L.javanica*

I = *L.icterohemorrhagiae*

- = not done

(i) The causative serotypes in 34 leptospirosis cases:

29 *L.bataviae*, 2 *L.canicola*, 2 *L.javanica*
1 *L.icterohemorrhagiae*

(ii) Positive hemocultures (21)

17 *L.bataviae*, 2 *L.canicola*, 2 *L.javanica*

(iii) Diagnosis by serology only (13)

12 *L.bataviae*, 1 *L.icterohemorrhagiae*

Summary and conclusion

Thirty four cases of leptospirosis in three hospitals in Bangkok were clinically studied. Most of them were admitted into the hospitals during late rainy season. About two-third were within the 20 - 39 - years age-group; the ratio of males to females was 2.4 : 1.

The symptoms and signs consisted of high fever, headache, myalgia, injected eyes, anorexia, nausea, vomiting, jaundice, enlargement of liver, etc. There were leucocytosis, increase in blood N.P.W. and changes in the urine findings.

Most cases were caused by L.bataviae. The mortality rate was nil.

4. Surveys of leptospiral antibodies in the general population of Thailand.

The results of detection of leptospiral antibodies in the general population of Bangkok and Chiangmai (as seen in our progress report, first part of the first year, pages 7-9) encouraged us to extend our study into those in other provinces of Thailand.

Materials and Methods

Blood specimens were taken from non-febrile adult patients in all 71 provincial hospitals (those in each hospital represented "the general population" of that province). In 24 provinces studies were made on fresh sera, and in the remaining 47 provinces dried blood samples on filter paper were used (a modification of Van Thiel et al*, 1963). 0.5 mL. of blood was dropped on Whatman No.4 filter paper, 3 by 8 cm., dried for 1 hour at room temperature, and mailed in an envelope to the Bangkok Laboratory where the paper was cut into several pieces and placed in a test tube containing 12.25 ml. of sterile isotonic saline. The serum was extracted by stirring with a glass rod at room temperature for one hour. After centrifugation, the supernatant fluid represented a solution of serum of 1:50 dilution (assuming the serum is about half of the whole-blood volume). The agglutination test with live antigens was performed on the fluid at dilutions of 1:100, 1:300 etc., using 10 leptospiral serotypes known to exist in Thailand, i.e. L.bataviae, L.icterohemorrhagiae, L.hebdomadis, L.autumnalis, L.grippotyphosa, L.canicola, L.javanica, L.pomona, L.tyrogens, and L.australis A. A titre of 1:100 or higher was considered to signify previous infection.

* Van Thiel, P.H., Van Der Hoeven, J.M. and Couree, L.M.J. (1963)

Results

The incidence of seropositive individuals varied from 22% in Central Thailand to 35% in South Thailand (Table VIII). The common serotypes were L.grippytyphosa, L.icterohemorrhagiae and L.bataviae (Table IX), which accounted for 69% of the total positive sera. Most of the seropositive subjects were farmers (Table X). Only 243 (35%) out of 689 seropositive cases had history of fever in the last two years. Since leptospiral agglutination titres usually fell to about zero within 2 years after the infection (Sundharagiati & Buspavanich*, 1951), this suggested that about 2/3 of our cases suffered from symptomless leptospirosis.

Table VIII. Results of leptospiral antibodies in the general population of Thailand.

Part of Thailand	No. of Provinces	Male			Female			Both sexes		
		No. exam.	No. pos.	% pos.	No. exam.	No. pos.	% pos.	No. exam.	No. pos.	% pos.
Central	19	504	117	23	572	120	20	1076	237	22
North	15	369	127	34	418	94	22	787	221	28
South	15	375	143	38	290	89	30	665	232	35
Northeast	15	428	139	32	362	79	22	790	218	28
East	7	235	78	33	193	42	22	428	120	28
Total 5 parts	71	1911	604	42	1835	424	23	3746	1028	27

* Sundharagiati, B., & Buspavanich, S. (1951) J. med. Ass. Thailand 34, 1.

Table IX Details of the total positive serotypes in the general population of Thailand

Serotype	Area					Total	
	Central	North	South	North-east	East	No.	%
<i>L.grippotyphosa</i>	49	64	72	64	13	262	25.5
<i>L.icterohemorrhagiae</i>	54	62	58	49	16	239	23.2
<i>L.bataviae</i>	51	32	49	33	44	209	20.3
<i>L.hebdomadis</i>	7	20	14	13	16	70	7.0
<i>L.javanica</i>	14	12	6	15	10	57	5.0
<i>L.autumnalis</i>	17	12	2	13	9	53	5.0
<i>L.canicola</i>	23	3	10	3	6	45	4.5
<i>L.australis A</i>	7	8	8	15	4	42	4.5
<i>L.pomona</i>	6	4	8	6	2	26	2.5
<i>L.pyrogenes</i>	9	4	7	7	0	25	2.5

Table X The occupation of all positive leptospiral cases in the general population of Thailand

Occupation	Area					Total	
	Central	North	South	North-east	East	No.	%
Farmer	94	67	76	91	36	364	52.5
Labourer	22	17	35	7	14	95	13.8
House wife	19	10	17	9	10	65	9.2
Merchant	12	9	12	11	15	69	8.5
Gardener	7	2	31	0	11	51	7.3
Officer	7	7	2	10	2	28	4.0
Fisherman	10	0	3	0	1	14	2.0
Bank	5	2	0	2	1	10	1.4
Student	1	0	3	3	0	7	1.3

Summary and Conclusion

Positive agglutinations reacted to various serotypes of Leptospira were found in 22-35 per cent of the general population of Thailand. The highest incidence occurred in the South. The common serotypes were L.grimotiphosa, L.icterohemorrhagiae and L.bataviae.

5. Detection of Leptospirosis in cases of pyrexia of unknown origin

During the past 12 months, September 1964-August 1965, an attempt to diagnose leptospirosis in F.U.O. cases was made in the Medical Department of Vajira Hospital (a Bangkok municipality hospital).

Materials and Methods

All F.U.O. cases attending the Medical Department of Vajira Hospital (most cases being investigated in the wards after admission; some investigated at the out-patient clinic) were studied by hemocultures and serological means to accomplish for the diagnosis of leptospirosis. Fletcher's medium was used for hemoculture. Agglutination with live antigen were used for serological tests and 11 serotypes* of leptospires known to exist in Thailand were used as antigens, i.e. L.bataviae, L.icterohemorrhagiae, L.canicola, L.shivami A., L.javanica, L.pyrogenes, L.gripotyphosa, L.pomona, L.australis A., L.hebdomadis and L.hyds. The conclusive diagnosis based on positive hemoculture and/or significant serological titres of paired sera. A titre of 1:100 or higher was considered to be significant.

Results

1. Total cases studied 228, 147 males and 81 females.
2. Admission, 159 cases; out-patient clinic, 69 cases.
3. Positive hemoculture in 9 and positive serologically (rising titres) in 25 (a total of 34 cases = 15% of all cases studied).

* Formerly we used 10 serotypes, but later on we found one more serotype (L.hyds) in some provinces of Thailand.

4. Analysis of those 34 positive cases revealed

(i) Age

<u>Age in years</u>	<u>No. of cases</u>
0 - 9	1
10 - 19	4
20 - 29	5
30 - 39	9
40 - 49	11
50 - 59	3
60 and over	1

(ii) Sex

25 males, 9 females

(iii) Occupation

<u>Occupation</u>	<u>No. of cases</u>
Labourer	15
Officer	4
Merchant	4
Gardener	3
House wife	3
Student	3
Farmer	2

(iv) Provisional diagnosis

	<u>No. of cases</u>
lyrexia, cause ?	11
Leptospirosis ?	10
Jaundice, cause ?	5
Influenza	5
Infectious hepatitis	1
Tonsillitis and pharyngitis	2

(v) Seasonal incidence

Table XI Showing seasonal incidence of 34 cases of leptospirosis

Month	No. of cases exam.	No. of cases positive	% positive
September	17	3	17.7
October	21	5	23.8
November	51	14	27.5
December	29	4	13.8
January	18	1	5.6
February	14	0	0
March	21	0	0
April	16	2	12.3
May	11	0	0
June	10	1	10.0
July	6*	2	-
August	14	2	14.3

* The number of cases was too small.

During the wet season (June-October), 68 cases were examined and 13 (19.1%) gave positive results, while during the dry season (January-May) 80 cases were examined and 3 (3.7%) were positive. Thus the incidence of the disease in the wet season was five times greater than that in the dry season.

(vi) Serotypes involved

18 L.bataviae, 9 L.canicola, 4 L.javanica, 2 L.icterohemorrhagiae, 1 L.grippotyphosa.

Summary and Conclusion

Leptospirosis is considered to be a cause in P.U.O. cases. Our results revealed that about 15% of those cases suffered from leptospirosis.

The clinical and laboratory features of 34 leptospirosis cases were studied in detail. The common causative serotypes were L.bataviae and L.canicola. L.javanica and L.icterohemorrhagiae were also found in many patients.

The incidence of the infection was high in the wet season, about 5 times as much as that in the dry season.

6. Macroscopic screening test of leptospirosis

Attempts have been made to obtain quick and reliable results of agglutination test which can be simply accomplished by reading with naked eyes or hand lens. The method described by Galton et al* was tried in our laboratory with satisfactory result. The antigens were made of formalinized individual leptospiral serotype at cocentrations of 1,000-2,000 million per ml. One drop of the antigen was mixed with 0.01 ml. of the unknown serum on a glass plate and rotated on a mechanical rotator for 5 minutes, after which the agglutination was easity read on translumination box, and the results were expressed as 1⁺, 2⁺, 3⁺ and 4⁺ by using naked eyes or hend lens.

The results are shown in Table XII - XVII

Table XII Macroscopic screening test using homologous antigens

Known sera		<u>Leptospira</u> used as antigen	Positive Results	
Number	Against <u>Leptospira</u>		No.	%
125	bataviae	bataviae	113	90.4
26	javanica	javanica	15	93.8
15	canicola	cenicola	15	100.0
2	australis A	australis A	2	100.0

* Galton M.M., Powers D.K., Hall A.D. & Cornell, R.G. (1958)

Amer. J. of Vet. Res. 19. 505

Table XIII Macroscopic screening test using various heterogenous antigens

Known sera		<u>Leptospira</u> used as antigen	Positive Results	
Number	Against <u>Leptospira</u>		No.	%
125	batavine	javanica	71	56.8
113	bataviae	akiyami A	65	57.5
113	bataviae	biflexa	8	7.1
125	bataviae	icterohemorrhagiae	10	8.0
14	javanica	bataviae	5	35.7
14	javanica	akiyami A	8	57.1
14	javanica	biflexa	5	35.7
14	javanica	ictero	1	7.1
14	canicola	bataviae	3	21.4
14	canicola	javanica	11	78.6
14	canicola	akiyami A	4	28.6
14	canicola	icterohemorrhagiae	1	7.1

Table XIV Macroscopic screening test using L.biflexa 136 and L.biflexa 138 as antigens

Known sera		<u>Leptospira</u> used as antigen	Positive Results	
Number	Against <u>Leptospira</u>		No.	%
12	bataviae	biflexa 136	6	50
12	bataviae	biflexa 138	11	91.7

Table XV Macroscopic screening test on sera of dogs with homologous antigen

Titre	<u>L.bataviae</u>		<u>L.ictero.</u>		<u>L.javanica</u>		<u>L.canicola</u>		<u>L.skiyamia</u>		Total	
	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve
1:100	16	4	19	0	4	0	2	0	3	0	44	4
1:300	5	1	1	1	-	-	1	0	-	-	7	2
1:1,000	5	2	-	-	-	-	2	1	-	-	7	3
1:3,000	4	4	-	-	-	-	1	1	-	-	5	5
Total	30	11	20	1	4	0	6	2	3	0	63	14

Table XVI Macroscopic screening test on sera of rats with homologous antigen

Titre	<u>L.bataviae</u>		<u>L.javanica</u>		<u>L.ictero.</u>		Total	
	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve
1:100	53	12	16	4	1	0	70	16
1:300	9	7	1	0	-	-	10	7
1:1,000	7	6	-	-	-	-	7	6
1:3,000	2	2	-	-	-	-	2	2
Total	71	27	17	4	1	0	89	31

Table XVII Macroscopic screening test on sera of swine with homologous antigen

Titre	<u>L.pomona</u>		<u>L.ictero.</u>		<u>L.bataviae</u>		<u>L.canicola</u>		<u>L.akiyama</u>		Total	
	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve	No. exam.	No. +ve
1:100	11	0	10	1	3	0	2	0	1	0	27	1
1:300	-	-	-	-	-	-	-	-	1	0	1	0
Total	11	0	10	1	3	0	2	0	2	0	28	1

Summary and Conclusion

The study of macroscopic screening test as mentioned above is only a preliminary step towards the simple procedure for diagnosis of leptospirosis. This method will be very useful to the provincial hospitals where there are inadequate laboratory facilities.

For human leptospirosis this method was very satisfactory even though the cases had a low titre of 1:100. In animal leptospirosis, the test was not so sensitive; only sera specimens with high titres gave some positive reactions.

The next step of our study in this particular subject will be the improvement of antigen preparation, especially in preparing L.biflexa 138 antigen. This organism had a promising antigenic property reacting with L.bataviae antisera. The antigen will be useful for the provincial hospitals in screening suspected cases of leptospirosis before sending the dried blood on filter paper to our laboratory in Bangkok.

B. Leptospirosis in animals

1. The incidence of leptospirosis in rats in Bangkok.

Studies on leptospirosis in rats caught from ten districts of Bangkok were carried out during April 1963 and June 1964; 206 (34.1%) of total 604 rats examined showed positive results (Boonpacknavig et al* 1965). However, the work has been carried on with special attention to Pathumwan District, where the incidence of leptospirosis among R.norvegicus rats (the most common species) was high. The seasonal variation was also studied.

Results

From September 1964 through August 1965, 648 R.norvegicus, including 467 adults and 181 immatures, were trapped and examined. The results of kidney cultures and serological examinations are shown in Table XVIII & XIX.

* Boonpacknavig, S., Harinasuta, C. and Pottha, U. (1965) J. med.

Ass. Thailand 1965 48, 352.

Table XVIII The incidence of leptospirosis in adult and immature rats of Pathumwan District according to seasonal variation.

Month	Adults					Immatures				
	No. exam.	Positive culture		Positive sera		No. exam.	Positive culture		Positive sera	
		No.	%	No.	%		No.	%	No.	%
January)	31	23	74.2	6	19.4	13	1	7.7	1	7.7
February)	21	11	52.4	6	28.6	22	2	9.1	2	9.1
March) Dry	37	25	67.6	16	43.2	30	1	3.3	2	6.7
April)	39	14	35.9	15	38.5	24	4	16.7	1	4.2
May)	26	19	73.1	4	15.4	3	0	0	1	33.3
June)	57	34	59.6	19	33.3	27	4	14.8	3	11.1
July)	42	24	57.1	16	38.1	12	1	8.3	0	0
August) wet	39	21	53.9	13	33.3	11	2	18.2	2	18.2
September)	24	19	79.1	14	58.3	8	0	0	0	0
October)	50	39	78.0	16	32.0	11	1	9.1	1	9.1
November	55	45	81.8	23	41.8	11	0	0	0	0
December	46	37	80.4	24	52.2	9	4	44.4	3	33.3
Total	467	311	66.6	172	36.8	181	20	11.5	16	8.9

During the dry season (January-May) 154 adult R. norvegicus were examined and 92 (59.7%) were positive for kidney culture, while during the wet season (June-October) 212 adult rats were examined and 137 (64.6%) were positive. Thus the leptospiral infection among the adult rats in the wet season was higher than that in the dry season.

Table XIX A comparison between the positives by culture and those by serology in adult rats

Animals	No. exam.	Positive both culture & sero.		Positive culture, negative sero.		Positive sero, negative culture		Negative both	
		No.	%	No.	%	No.	%	No.	%
Male	225	58	25.8	83	36.9	17	7.6	67	29.8
Female	242	76	31.4	94	38.8	20	8.3	52	21.5
Total	467	134	28.7	177	37.9	37	7.9	119	25.5

Summary and Conclusion

The incidence of leptospirosis diagnosed by kidney culture among adult rats (66.6%) was much higher than that of immature (11.5%). There was no difference in the incidence between sexes. Only 134 (43.1%) out of 311 positive kidney culture rats had leptospiral antibodies.

The serotypes of those leptospires isolated were identified as 256 L.batavine and 36 L.javanica. Other isolates are being studied.

2. Seasonal incidence of canine leptospirosis in Bangkok

The incidence of canine leptospirosis in Bangkok has been reported (Sundharagisti et al 1965)*. However, we have continued our study with special attention to its seasonal variation.

Materials and Methods

The diagnosis of leptospirosis in dogs was made by the agglutination (lysis) test, using serum from the heart blood. The titres of 1:100 - 1:1,000 were considered as signifying previous infection, and 1:3,000 or higher as recent infection. 1022 dogs at the municipality stray dog-eradication unit in Bangkok were included in this study.

Results

The results are shown in Table XX. During January through May, when the weather was mostly dry and the humidity low, of all 358 sera examined, 183 (51.1%) were positive for leptospirosis at the titres of 1:100 - 1:1,000, and 9 (2.5%) had the titres of 1:3,000 or higher. During June through October, the wet season with high humidity, of all 528 sera examined, 330 (62.5%) were positive at the titres of 1:100 - 1:1,000, and 64 (12.1%) had the titres of 1:3,000 or higher.

Thus the incidence of recent infection of canine leptospirosis in Bangkok in the wet season was about 5 times greater than that in the dry season.

* Sundharagisti, B., Boonpachnavig, S. & Harinasuta, C. (1965)
Trop. geogr. Med. 1, 17.

Table XX The incidence of canine leptospirosis in Bangkok as diagnosed by serology.

Month	No. sera tested	Titres of 1:100 - 1:1,000 (previous infection)		Titres of 1:3,000 or higher (recent infection)	
		No.	%	No.	%
January)	44	14	31.8	1	2.3
February)	51	26	50.9	1	1.9
March) Dry	58	31	53.4	1	1.7
April)	60	24	40.0	1	1.7
May)	145	88	60.7	5	3.4
June)	168	98	58.3	7	4.2
July)	104	83	79.8	23	22.1
August) Wet	153	89	58.1	16	10.5
September)	50	29	58.0	6	12.0
October)	53	31	58.5	12	22.6
November	88	40	45.4	7	7.9
December	48	18	37.5	7	14.6
Total	1022	571	55.9	87	8.5

- The rates of recent infection were highest in July and October.

- Total positive rate = $55.9 + 8.5 = 64.4\%$

Summary and Conclusion

The sera of 1022 stray dogs in Bangkok were examined for leptospiral antibodies, and 64.4% gave positive results. The incidence of recent infection in the wet season was 12.1%, while that in the dry season was 2.5%. July and October were found to be the months of having high transmission of canine leptospirosis.

3. Studies on renal pathology in canine leptospirosis

This project has been carried out with the collaboration of the Departments of Pathology of S-ATO Medical Research Laboratory (Lt. Col. E.H. Johnston) and the Faculty of Medicine Chulalongkorn Hospital (Dr. P. Fiyaratn)

Materials and Methods

The materials were obtained from the dogs at the municipality stray dog-eradication unit in Bangkok. The heart blood and kidney tissue aspirates were taken from the dogs just after death. These specimens were bacteriologically and serologically studied to determine the presence of leptospiral infection. Fletcher's medium was used for kidney culture. Serological investigations which included agglutination tests with live antigens were made against the following serotypes: i.e. L.bataviae, L.icterohemorrhagiae, L.canicola, L.hebdomadis, L.akiyami A, L.javanica, L.grimotyrphosa, L.australis, L.pomona, L.pyrogenes and L.hyos. A titre of 1:100 or higher was considered significant.

Specimens of both kidneys from each dog were examined grossly. A slice of the whole kidney from each specimen was fixed in 10 per cent buffered formalin solution. Tissue blocks were taken from each slice; one from the upper pole, one from the lower pole and one from the middle. Sections were stained by hematoxylin-eosin and PAS method. In addition, at least one slide from each case was stained by Warthin-Starry method to demonstrate leptospiral organism; the experimentally infected tissue of hamster was also used as control.

Results:

Primarily 40 dogs were included in this study, but later 9 had to be discarded because of contamination. The remaining 31 cases were thus divided into three groups as follows:-

Group I - Positive culture (3 dogs)

<u>Dog No.</u>	<u>Organism</u>	<u>Titre</u>
915	L.bataivae	negative
918	L.canicola	1:3,000
939	L.javanica	1:100

Group II - Positive serology (7 dogs)

<u>Dog No.</u>	<u>Serotype</u>	<u>Titre</u>
902	L.bataviae	1:100
919	L.javanica	1:100
932	L.bataviae	1:300
933	L.canicola	1:100
934	L.bataviae	1:100
936	L.bataviae	1:300
940	L.hyo	1:100

Group III - Negative culture and negative serology

Twenty one dogs.

Pathological studies of the kidney tissue were concentrated on the so-called "interstitial nephritis" which had been previously described as characteristic pictures in the dog infected with L. canicola. The preliminary results in our investigation are as follows:-

Group I

Grossly, the kidney showed scattered small gray to yellow nodules throughout the cut surface and also on the external surface. The most pronounced lesion was seen in case # 918 from which L. canicola was isolated. Histologically, all three cases had similar pathology but of varying intensity. The one with much severe changes was case # 918 as grossly evident. The lesion consisted of inflammatory foci scattered in both cortex and medulla of the kidney. Most of the cellular exudate was in the interstitial tissue surrounding convoluted tubules and also collecting tubules. Occasionally, however, the tubular epithelial cells in the central portion of the foci showed degenerative changes and necrosis with intratubular cell infiltration. Basement membrane, as shown by PAS stain, particularly of the damaged tubules was broken or frayed. The infiltrating cells comprised of neutrophils, lymphocytes, histiocytes and plasma cells in varying proportions. Glomeruli were not remarkable as well as other vascular structures. Leptospire were demonstrated in tubules of few areas in these three dogs.

Group II

No active lesions similar to those found in group I were observed in all cases. However, interstitial inflammatory foci were constantly present. The extensiveness of the lesions varied from slight to moderate. Foci were found in both cortex and medulla. No tubular damage was evident.

Peritubular infiltration consisted preponderantly of plasma cells, and in lesser degree lymphocytes and histiocytes. Fibrous proliferation in these foci varied from nothing to marked. Leptospire could not be demonstrated in all cases.

Group III

Out of 21 cases, 6 showed no interstitial inflammation; the remaining 15 revealed changes similar to those observed in group II. The severity of the lesion was slight in all except one which was moderate. The cellular infiltrates comprised of lymphocytes and plasma cells and rarely eosinophils. Fibrous proliferation of the inflammatory foci, which varied from slight to marked, was evident in 10 cases. Leptospire could not be demonstrated in all cases.

Summary and Conclusion

The primary results of the study on renal pathology of 31 stray dogs in Bangkok were satisfactory. In the group of positive kidney culture, the pathological changes consisted of the inflammatory foci scattered in both cortex and medulla of the kidney; some degenerative changes and necrosis were noticed in tubular portions. In the group of positive serology, no active lesions were observed, but interstitial inflammatory foci were constantly present. In the group of no evidence of leptospirosis, slight interstitial inflammation was noted in many animals.

The work is still in progress.

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13. ABSTRACT <u>Studies on leptospirosis, with special reference to the epidemiology, pathology and clinical aspects, and its relation to the animal reservoir hosts.</u> Epidemiological study on leptospirosis in Thailand by surveying leptospiral antibodies among the general population in 71 provinces revealed positive agglutination reactions of 22-35%. The high incidences were mostly found in South Thailand. The common serotypes were <u>L. grippotyphosa</u> , <u>L. icterohemorrhagiae</u> and <u>L. bataviae</u> . Most cases were farmers; the ratio of males to females was 3:2. Examinations for laboratory diagnosis of leptospirosis were made in the past twelve months in about 1400 patients of various hospitals throughout Thailand. The results showed the <u>L. bataviae</u> was the most prevalent serotype in Bangkok, while <u>L. grippotyphosa</u> was the common one in the provincial areas. The clinical and laboratory features of 34 cases of leptospirosis, most of which caused by <u>L. bataviae</u> , were also analysed. About 15% of cases of pyrexia of unknown origin in a municipality hospital of Bangkok were found to suffer from leptospirosis, and <u>L. bataviae</u> and <u>L. canicola</u> were the common serotypes involved. The results of macroscopic screening tests in human leptospirosis were satisfactory, while in animal leptospirosis only the specimens with high titres gave some positive reactions. <u>L. biflexa</u> No. 138 had a promising antigenic property reacted to <u>L. bataviae</u> antisera.			

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Studies on leptospirosis in rats caught from Pathumwan District of Bangkok revealed positive kidney-culture cases of 67% in adult rats and 12% in immature ones. The incidence among the adult rats in the wet season was 65% and in the dry season 60%.

The incidence of canine leptospirosis in Bangkok diagnosed by serology was found to be 64%. The results also indicated that recent infections in dogs were present mostly in the wet season.

The preliminary results of the studies of renal pathology in canine leptospirosis revealed definite pathological changes in cases of positive kidney cultures, while in cases of positive serology, only mild grades of inflammatory foci were observed. In those negative cases, however, slight histological changes were found in some animals. (Author)